A method of rapid Rh typing employing the capillary tube technique and papainized incomplete anti-D sera

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The rapid capillary tube method for Rh typing introduced by Chown (1944) has the virtues of simplicity and of requiring the minimum of time, materials, and equipment, but has the disadvantages of requiring highly selected, potent and avid sera of the saline-agglutinating type. The following modification of the capillary tube technique, employing papainized incomplete anti-D sera, appears to have all the advantages of the original Chown technique without the disadvantage of having to use specially selected sera in order to reach a high standard of accuracy.

MATERIALS USED

ANTI-D SERA Two incomplete anti-D sera were used routinely in albumin as typing reagents. In addition 20 random incomplete anti-D sera, supplied by the Blood Group Reference Laboratory, all of which were found to be suitable as test reagents using the albumin replacement technique, were examined to determine the proportion which would be suitable for the modified capillary tube method.

PAPAIN SOLUTION Activated papain was used, made according to the method of Löw (1955).¹ For convenience, these details are repeated. Two grams of papain are ground in a mortar with 100 ml. M/15 phosphate buffer, pH 5.4. After filtration, 10 ml. 0.5 M cysteine is added to activate the enzyme. The solution is then diluted with the buffer of 200 ml. and incubated at 37°C. for one hour. It is then ready for immediate use.

The papain solution was stored at -20°C. in small amounts sufficient for one day's use. Any unused at the end of the day was discarded and not re-frozen. This preparation has maintained its activity for more than a year and no significant deposit or turbidity has occurred.

CAPILLARY TUBES These were obtained commercially and have an internal bore of 0.5 to 1.0 mm. and a length of 8.0 cm.

METHOD

Papain solution, 0.03 ml., was added to 0.03 ml. of the incomplete anti-D serum under test, mixed in a precipitin

¹In his original method Löw used Papayotin Merck 1:350, but the papain supplied by L. Light & Co., of Poyle Estate, Colnbrook, Bucks, was found to be perfectly satisfactory.

Received for publication 23 December 1960.

tube and the mixture immediately run into capillary tubes in approximately 0.02 ml. amounts. Taking care to avoid air bubbles, an equal amount of a 20 to 25% saline suspension of once washed test red cells was run into each capillary with the serum-papain mixture. The ends of the capillary tubes were then plugged with plasticine, set in a rack at an angle of 45° with the red cell suspension uppermost, and placed in an incubator at 37°C. for 15 minutes. The pattern of the sedimenting column of red cells followed that obtained in the original Chown technique, *i.e.*, the D-positive cells showing a granular or 'beaded' appearance and the D-negative cells showing a smooth unbroken sediment. Incubation beyond 15 minutes had no advantage, although, depending on the test serum, positive results were noted in a shorter time, with a few sera in as short a time as five minutes.

EXPERIMENTAL TRIALS

The standard Rh typing method in this laboratory employs albumin-agglutinating anti-D sera, a tube technique, and an incubation time of two hours. This method was used in parallel with the modified capillary tube technique on 500 consecutive routine blood samples submitted to the laboratory for grouping. The results of this trial are shown in Table I.

TABLE I
RESULTS OF STANDARD RH TYPING METHOD

<i>Tube Test Positive and Capillary</i>	<i>Tube Test Negative and Capillary</i>	<i>Tube Test Negative and Capillary</i>	<i>Tube Test Positive and Capillary</i>	<i>Total</i>
<i>Tube Test Positive</i>	<i>Tube Test Negative</i>	<i>Tube Test Positive</i>	<i>Tube Test Negative</i>	
420	79	1 ¹	0	500

¹The cells in this case were subsequently shown to be Rh type D^u.

Although during the period of trial the modified capillary tube method was used by experienced technicians, staff with limited experience in the use of serological methods and some junior staff with no previous experience of serology at all, it will be seen that the correlation between the tube technique and the modified capillary tube method is very good.

In order to discover the availability of incomplete anti-D sera suitable for this method, 20 random incomplete anti-D sera which gave satisfactory results using the albumin replacement technique were each tested against five D-positive and one D-negative blood sample. The results of this experiment are given in Table II.

From the results of this limited trial it appears that only one serum out of the 20 tested would be unsuitable for use by the modified capillary tube method. This suggests that a large number of sera which are suitable for use when diluted with albumin or in the albumin replacement technique are equally suitable for use in the modified capillary tube method with a great saving of time under emergency conditions. In addition to the 20 random sera tested in this series, one commercially produced anti-D

TABLE II

RESULTS WITH ALBUMIN REPLACEMENT TECHNIQUE

Cells	1	2	3	4	5	6	7	8	9	10
O D positive	SP	SP	SP	SP	SP	SP	SP	D	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	D	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	D	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	—	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	D	SP	SP
O D negative	—	—	—	—	—	—	—	—	—	—
	11	12	13	14	15	16	17	18	19	20
O D positive	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
O D positive	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
O D negative	—	—	—	—	—	—	—	—	—	—

SP=strongly positive

D=doubtful

serum (Ortho slide-test anti-D) was tested and found to be satisfactory.

DISCUSSION

The principle of serum papainization has been applied by Hekker, Klomp-Magnee, Krijnen, and van Loghem (1957) to the development of a rapid slide technique for mass Rh typing. This method employs a papain solution rather different from that of Löw and uses larger volumes of serum than those needed for the capillary tube method. The authors report that over a series of 29,000 tests checked by the saline-albumin method no discrepancies were found using their papain-slide method. These workers also found the large majority of incomplete anti-Rh sera to be useful reagents. They reported that the papain solution employed tended to develop a white sediment and centrifuging was necessary before use. Apart from a faint opalescence which in no way interferes with the tests, no such difficulty has been encountered with the papain solution used in the present series of tests.

It has been reported by Löw (1955), and confirmed by Hekker and his colleagues (1957), that the serum-papain mixture loses its activity on storage and this has been my experience during these experiments. For optimum results the serum-papain mixture should be used immediately

after mixing and certainly not later than three to four hours.

For a rapid Rh typing method to be satisfactory it must be capable of a high degree of accuracy, suitable for use with easily available typing sera, simple to perform, and easy to interpret. It should be free from technical difficulties even in relatively inexperienced hands and should give reliable results in the shortest possible time. While no method is likely to be absolutely foolproof the modified capillary tube method described here appears to satisfy these criteria, differing from the original technique of Chown in the use of the papainized incomplete anti-D serum. In addition it has the merit of using very small quantities of serum.

SUMMARY

A capillary tube method is described for rapid Rh typing based on Chown's technique and employing incomplete anti-D sera with the addition of activated papain.

When compared with a standard tube technique the method gave only one discordant result over a series of 500 random samples. This discrepancy was in respect of a blood subsequently shown to be of Rh type D^u.

Nineteen out of 20 incomplete anti-D sera tested proved suitable as typing reagents by this method.

The technique is simple and can be performed by relatively inexperienced staff, and very small quantities of serum are needed.

I would like to thank Miss K. E. Boorman and Miss B. E. Dodd for carrying out independent tests on the method, Miss Joan Lindars for her help in the experimental work, and Drs. M. Lubran and J. G. Selwyn for advice and criticism. Thanks are also due to Dr. Mourant, of the Blood Group Reference Laboratory, for supplying the unselected anti-D sera and to Ortho Pharmaceutical, Ltd., for supplying the commercial serum tested.

REFERENCES

- Chown, B. (1944). *Amer. J. clin. Path.*, 14, Tech. Sect., 8, 114.
 Hekker, A. C., Klomp-Magnee, Willy, Krijnen, H. W., and van Loghem, J. J., Jr. (1957). *Vox Sang. (Basel)*, n.s. 2, 128.
 Löw, B. (1955). *Ibid.*, 5, 94.